

Supplementary Information

***In situ* visualization and dynamics of newly synthesized proteins in rat hippocampal neurons**

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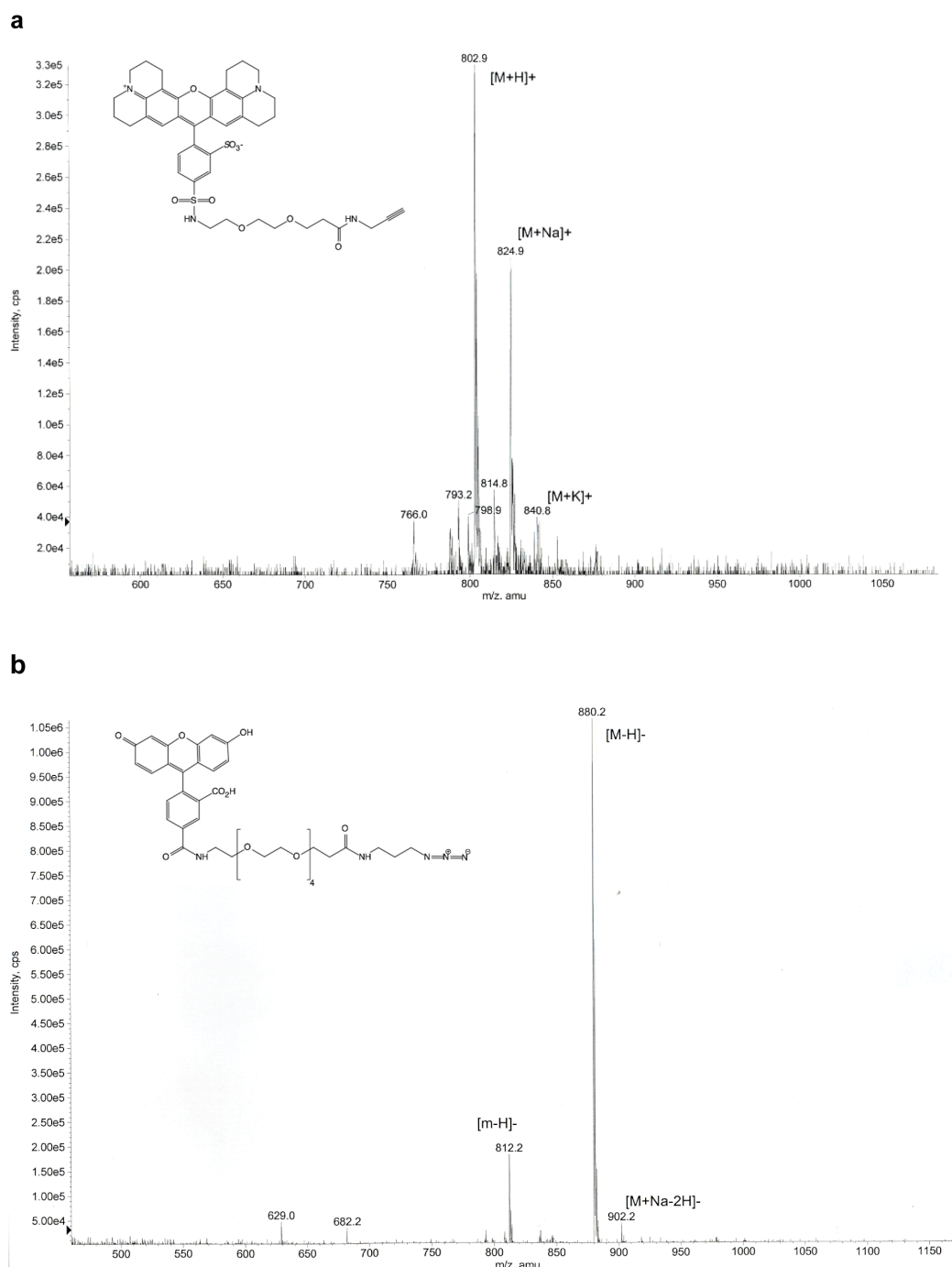
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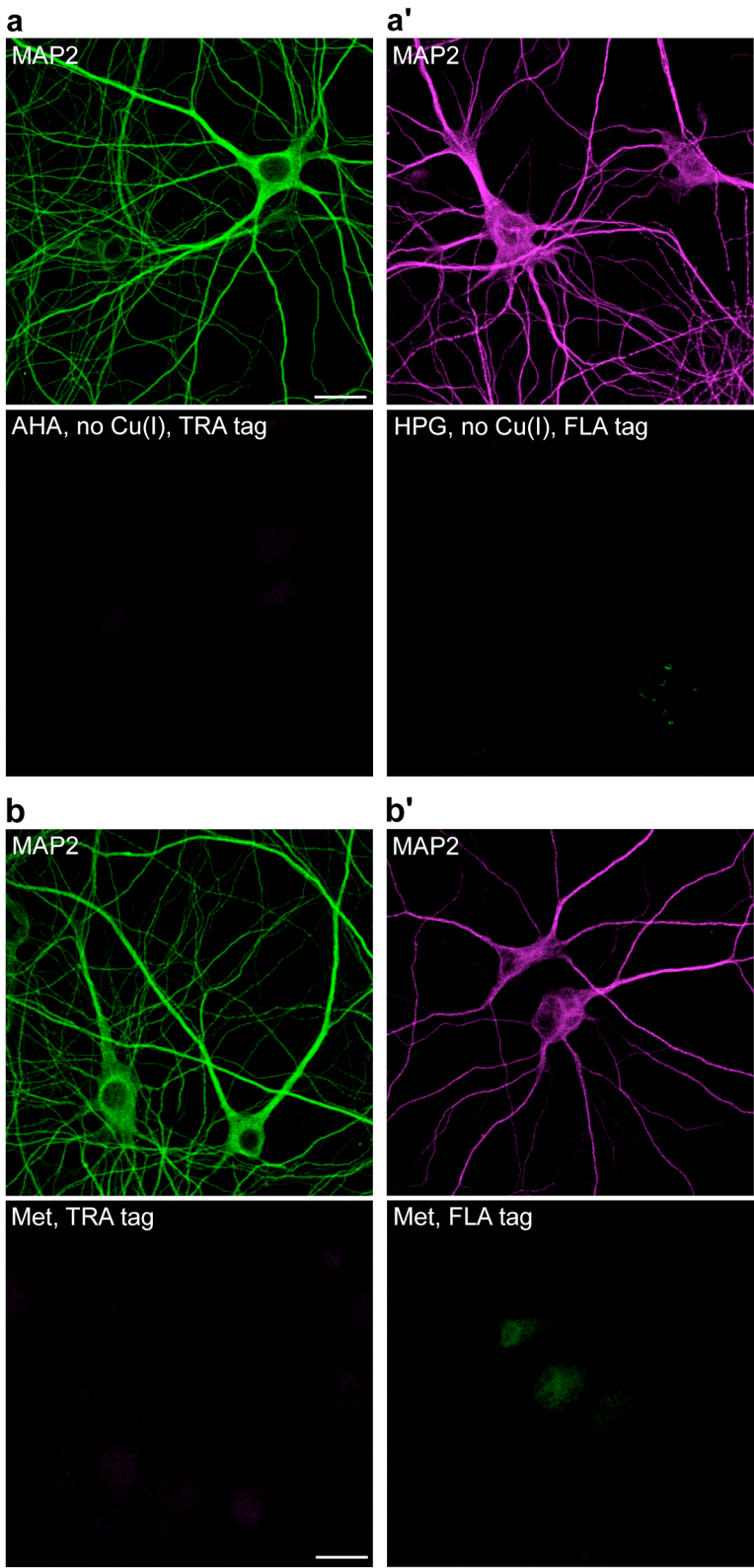
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Supplementary Figure 1



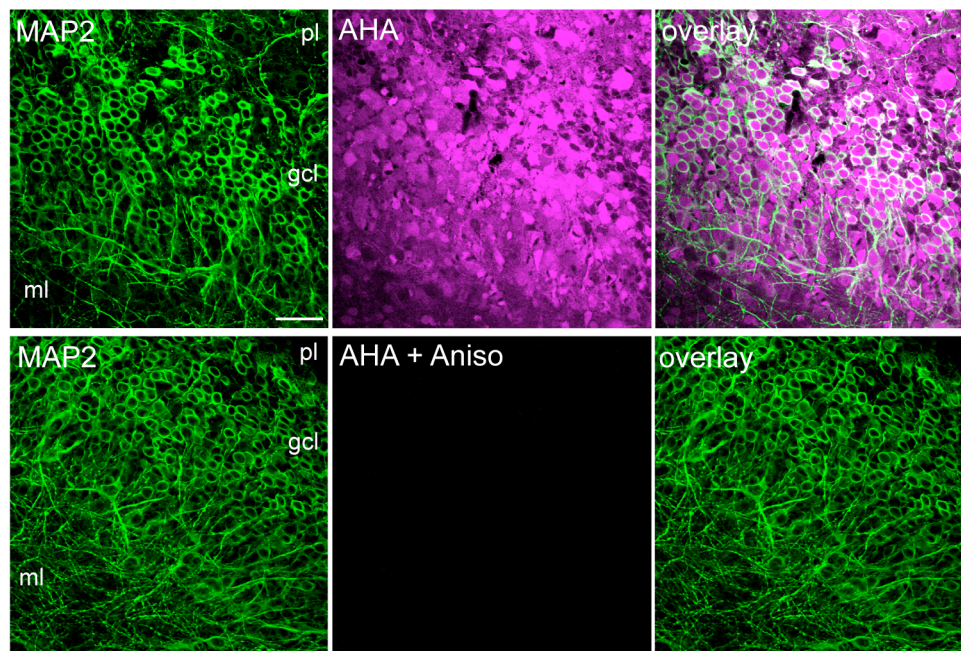
Analytical analysis of the fluorescent alkyne tags TRA and FLA. (a) Chemical structure and MS ESI analysis of the TexasRed-PEO₂-Alkyne tag (TRA). Calculated mass: 802.96, found: 802.90. (b) Chemical structure and MS ESI analysis of the 5-carboxyfluorescein-PEO₈-Azide tag (FLA). Calculated mass: 881.92, found: 881.20.

Supplementary Figure 2



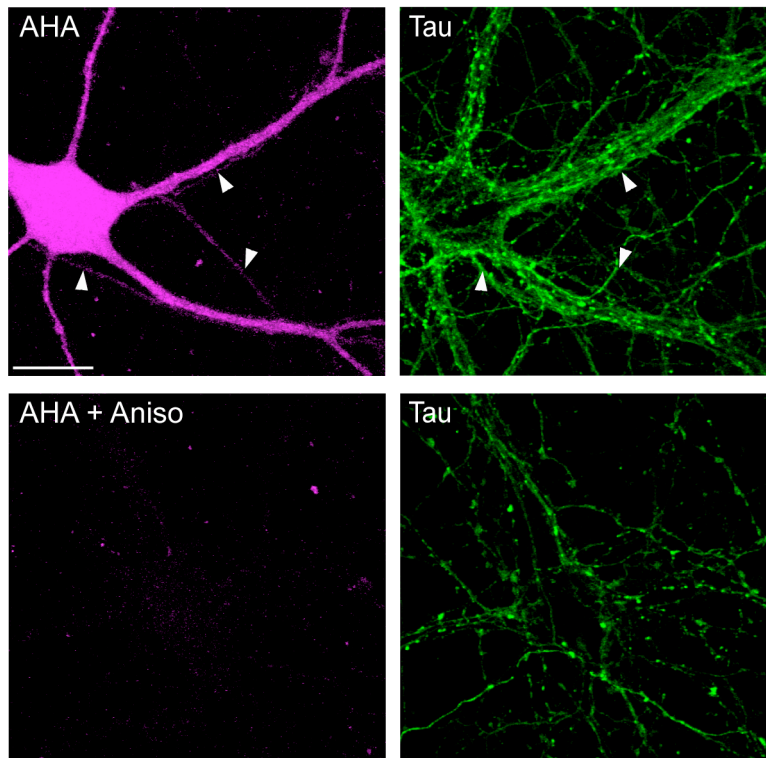
Specificity of CuAAC-mediated visualization of *de novo* synthesized proteins. (a,a') Dissociated hippocampal neurons (DIV 17) were incubated with either 2 mM AHA (a) or 2 mM HPG (a') for 1 h, tagged with 1 mM TRA or FLA tag in the presence or absence of the copper catalyst (CuSO₄) and immunostained for the dendritic marker protein MAP2. (b,b') Dissociated hippocampal neurons (DIV 17) were incubated with 2 mM Met for 1 h, tagged with either 1 mM TRA (b) or FLA (b') tag, and immunostained for the dendritic marker protein MAP2. Scalebar = 20 μm.

Supplementary Figure 3



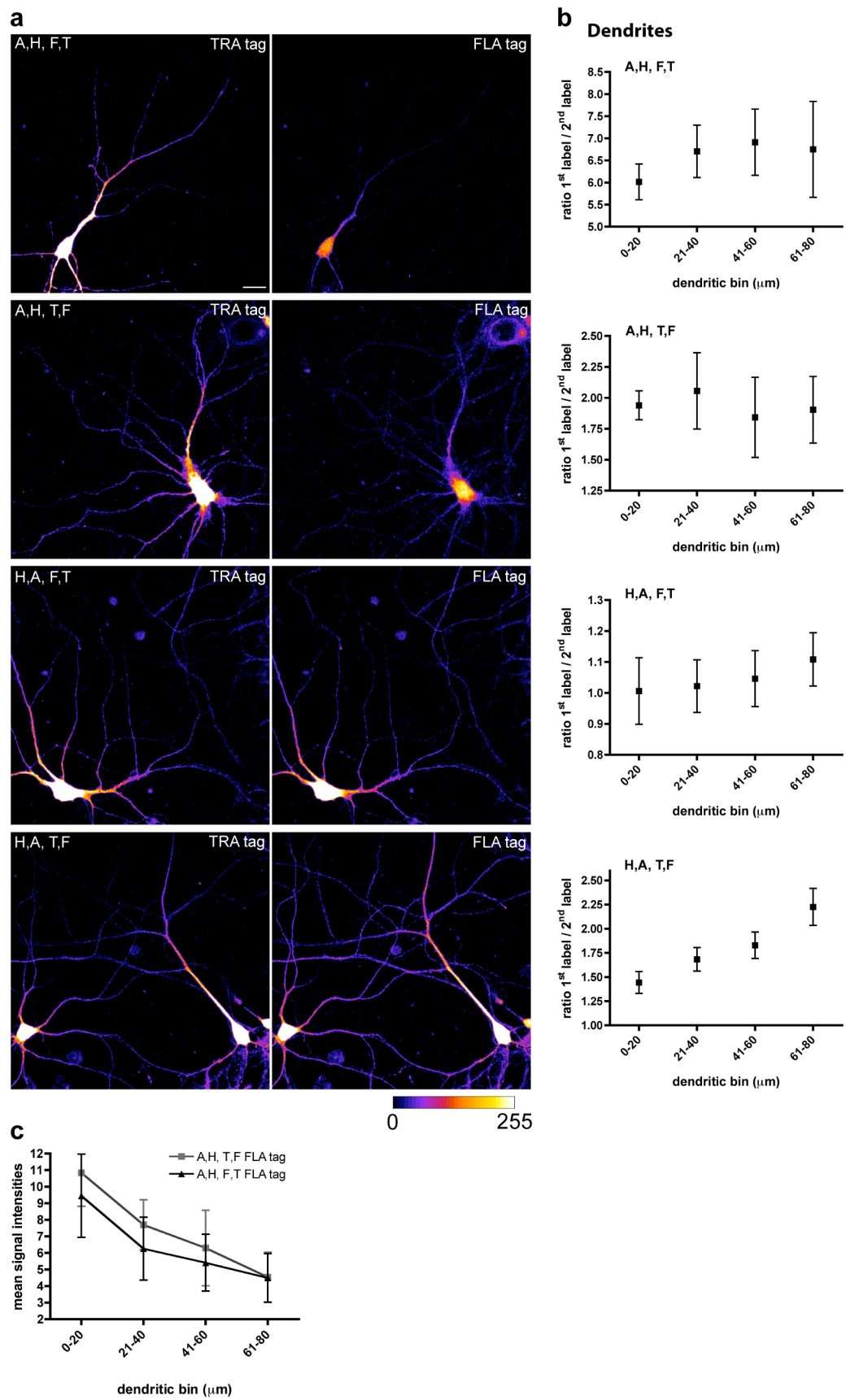
Visualization of newly synthesized proteins in organotypic hippocampal cultures. Organotypic hippocampal cultures (DIV 21) were incubated in 2 mM AHA in the presence or absence of 40 μ M anisomycin (Aniso) for 4 h, and tagged with 1 mM TRA overnight followed by MAP2 immunostaining. Images show the dentate gyrus with the granule cell layer (gcl), polymorphic layer (pl) and the molecular layer (ml). Scalebar = 50 μ m.

Supplementary Figure 4



Visualization of newly synthesized proteins in dissociated primary hippocampal neurons. Dissociated hippocampal neurons (DIV 17) were incubated with 2 mM AHA in the presence or absence of 40 μ M anisomycin (Aniso) for 3 h, tagged with 1 mM TRA or FLA tag and immunostained for the axonal marker protein Tau. Arrowheads denote axons. Scalebar = 20 μ m.

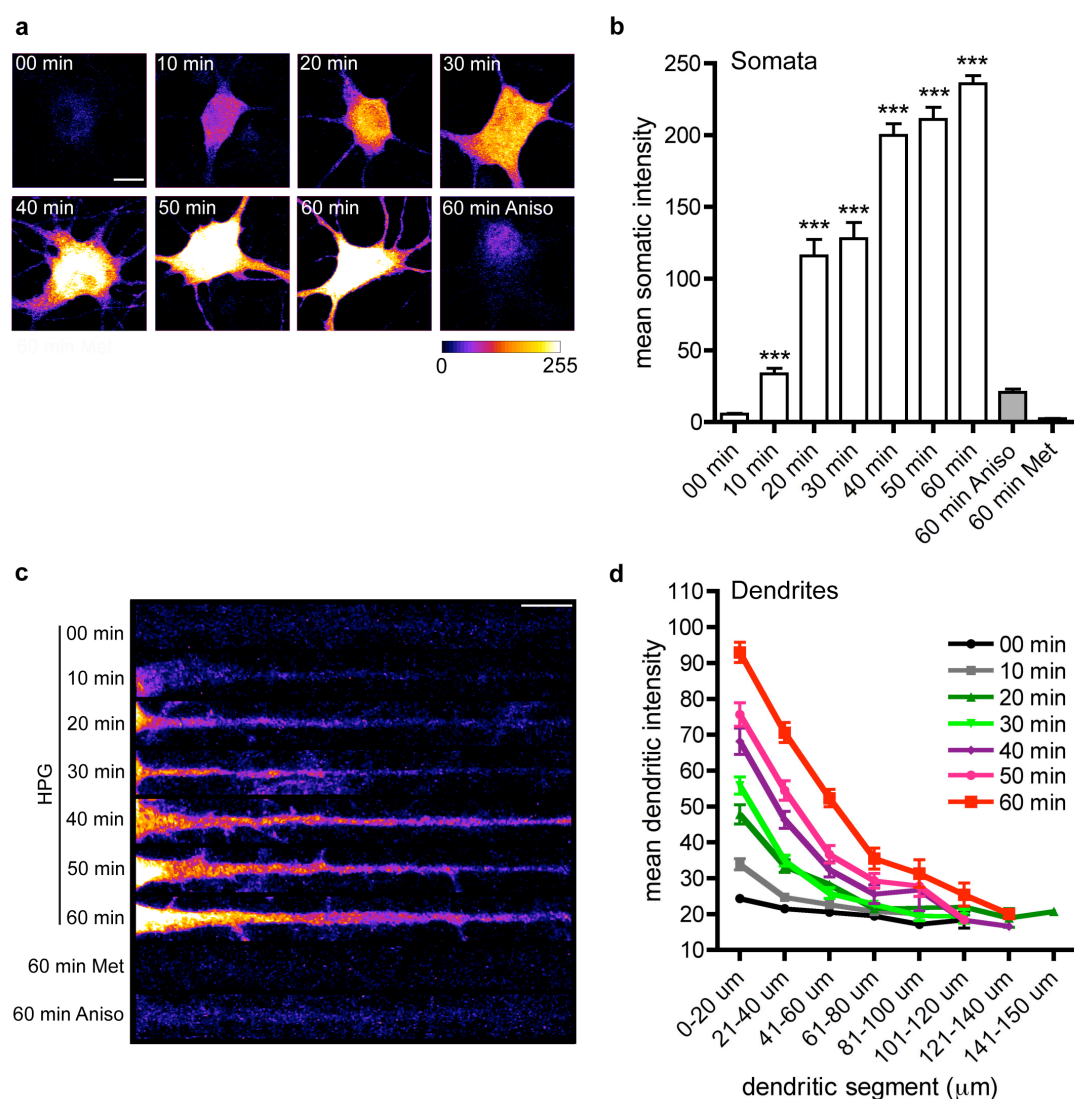
Supplementary Figure 5



Sequential labeling of two newly synthesized protein pools. (a)

Dissociated hippocampal neurons (DIV 16–18) were incubated in 2 mM AHA for 1.5 h followed by 4 mM HPG for 1.5 h and vice versa, and sequentially tagged with either 1 mM TRA then 1 mM FLA tag, or FLA tag followed by TRA tag for 12 h each. Scalebar = 20 μ m. Labeling and detection sequences: A,H, F,T: first AHA then HPG, first FLA then TRA tag; A,H, T,F: first AHA then HPG, first TRA then FLA tag; H,A, F,T: first HPG then AHA, first FLA then TRA tag; H,A, T,F: first HPG then AHA, first TRA then FLA tag. Images were acquired and analyzed using identical parameters on a Zeiss Meta 510 confocal microscope using a 40x objective; post-processing and analysis was done with ImageJ. Color lookup table indicates fluorescence intensity (pixel intensities 0–255). Graph (b) plots the mean ratio \pm SEM of the first to second label (AHA or HPG) intensity (\pm s.d.) of the dendrites in 20 μ m bins for the different amino acid and tag exposure sequences, n = 6–10 neurons. Graph (c) plots mean FLA tag signal intensities \pm SEM for A,H, F,T and A,H, T,F dendrites for dendritic bins indicated. n = 6–10 neurons.

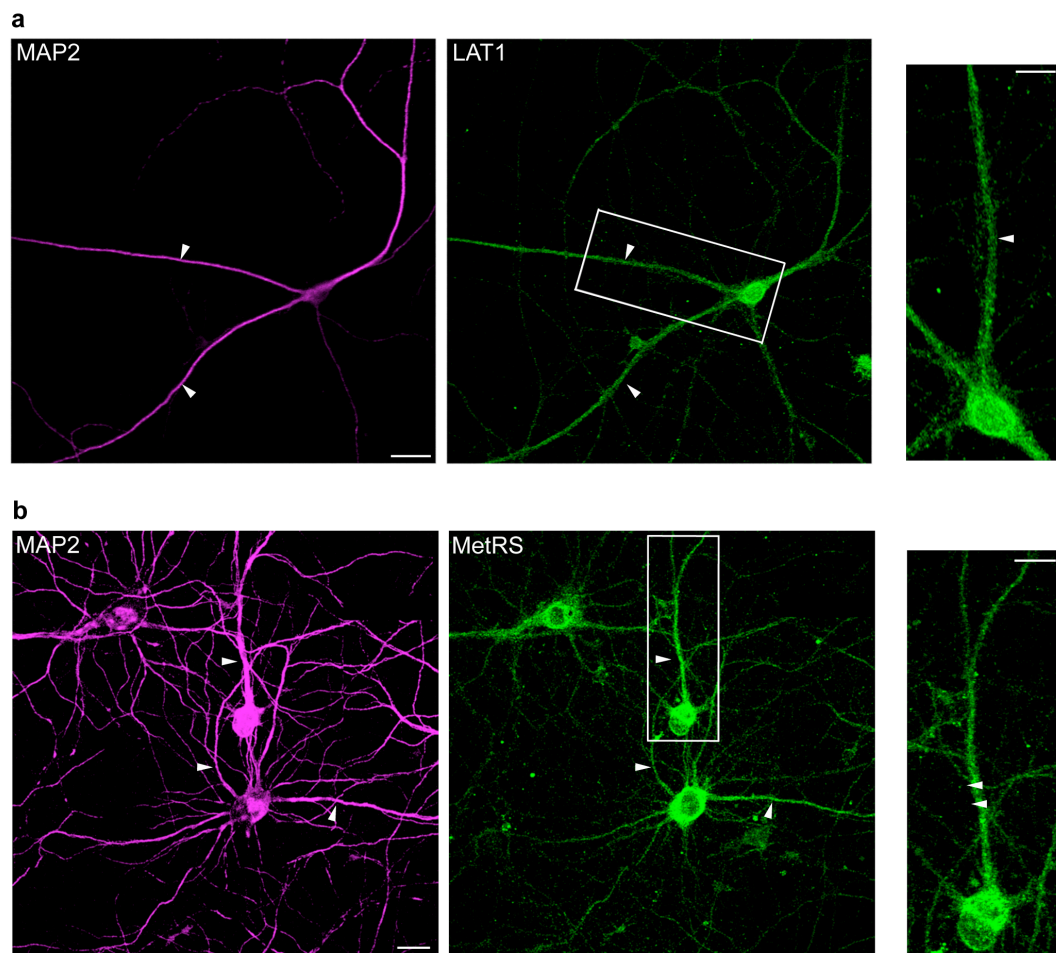
Supplementary Figure 6



Time-course for the detection of newly synthesized proteins in somata and dendrites. (a) Cultures (DIV 16) were incubated for the time points indicated with 2 mM HPG, 2 mM methionine (Met) or 2 mM HPG in the presence of 40 μ M anisomycin (Aniso). After cycloaddition with the fluorescent FLA tag, images were acquired with identical parameters on a Zeiss Meta 510 confocal microscope using a 63x objective. Scalebar = 5 μ m. Graph (b) represents mean intensities \pm SEM of the somata. Per time point, data from 20–50 cells were collected and analyzed using ImageJ. Representative examples are shown in the right panel. (c) Representative

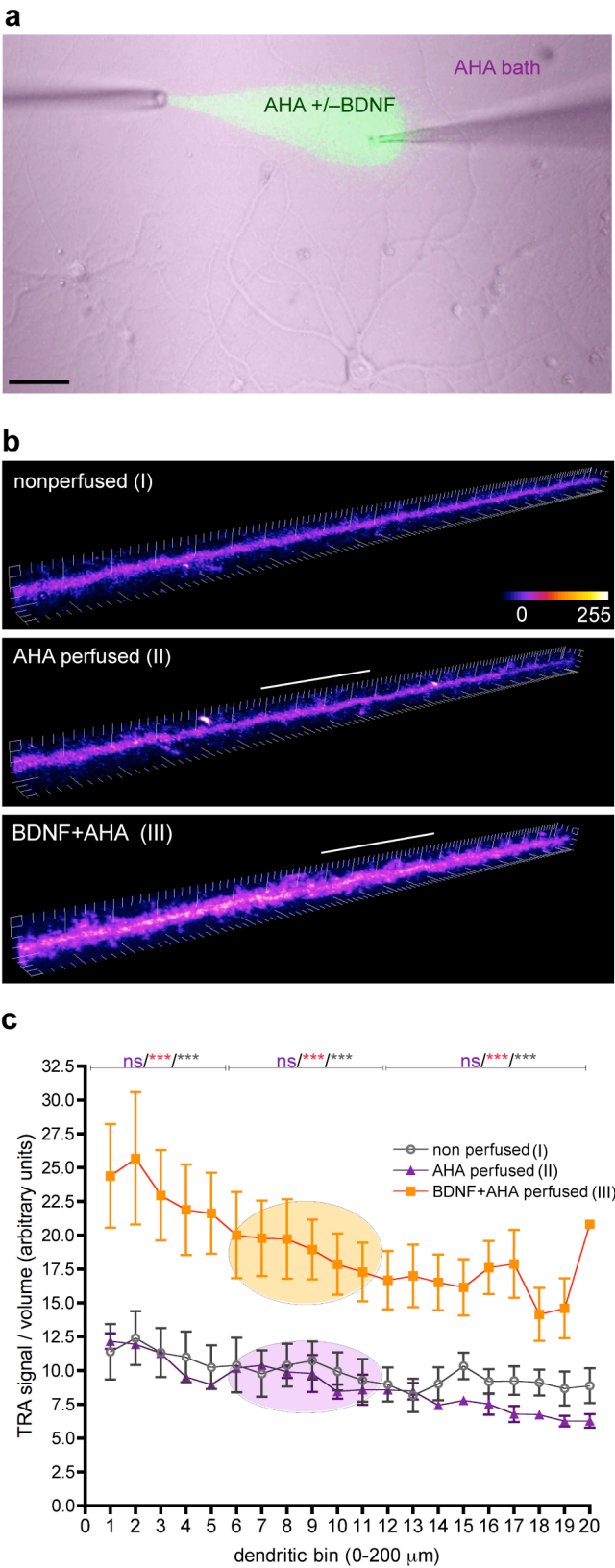
straightened dendrites of neurons incubated with 2 mM HPG, 2 mM Methionine (Met) or HPG plus 40 μ M anisomycin (Aniso) for time points indicated. Color lookup table indicates fluorescence intensity (pixel intensities 0–255). Left: proximal; right: distal. Scalebar = 10 μ m. Graph (d) represents mean intensities \pm SEM of the dendrites in 20 μ m bins. Per time point data from 30–70 dendrites were analyzed. Note, that somatic signals are saturated in some cases, in order to optimize the imaging parameters for signal detection in distal dendrite segments.

Supplementary Figure 7



The methionyl tRNA synthetase MetRS and the amino acid transporter LAT1 are present in dendrites. Immunostaining of dissociated hippocampal neurons (DIV 17–20) for both the dendritic marker protein MAP2 and the large amino acid transporter LAT1 (a), or methionyl tRNA synthetase (b). Arrowheads indicate dendritic processes. Scalebars = 20 μm (a) and 50 μm (b) in the overviews, scalebars = 10 μm in close-up images (a) and (b).

Supplementary Figure 8



Local BDNF-induced increase in dendritic protein synthesis. (a) Local dendritic microperfusion set-up showing a local perfusion stream (AlexaFluor

488 hydrazide in HibA) applied to a dendrite of a hippocampal neuron. Scalebar = 50 μ m. Neurons were pre-incubated in HibA media without methionine for 20 min during which time the micropipettes were positioned. Dendritic local perfusion with 2 mM AHA in the presence or absence of BDNF (50 ng/ml) in HibA was initiated immediately after AHA (2 mM) was applied to the bath solution. Dendritic microperfusion was performed for 30 min while constantly monitoring the perfusion stream. After immediate fixation, cells were processed for FUNCAT and MAP2 immunostaining, and imaged on a Zeiss Meta 510 confocal microscope using a 63x objective, post-processing and analysis was done with Imaris software. Images in (b) show TRA signals of representative dendrites from groups (I) no perfusion, (II) dendritic AHA perfusion, (III) dendritic BDNF plus AHA perfusion, in combination with AHA bath application. Grey bars mark the location and extension of the perfusion stream. Large grid tick marks = 10 μ m. Color lookup table indicates fluorescence intensity (pixel intensities 0–255). Graph (c) shows TRA–signal to volume ratios \pm SEM (based on MAP2 staining) of the representative dendrites. Colored ovals represent the positions of the perfusion spots. n= 4–5 dendrites. P-values: *** p < 0.001 using one-way ANOVA statistical analysis. Significances from Tukey post-hoc multiple comparison testing are indicated on top of graph for the dendritic segments before, within and beyond the perfusion spots; violet labels indicate significances for dendrites derived from group (I) vs. (II), red labels indicate significances for group (I) vs. (III) dendrites, grey labels indicate significances for group (II) vs. (III) dendrites.